

What Is Claimed Is:

1. A plant, the genome of which comprises introduced DNAs encoding the following enzymes:

5

a β -ketothiolase capable of condensing two molecules of acetyl-CoA to produce acetoacetyl-CoA and a β -ketothiolase capable of condensing acetyl-CoA and propionyl-CoA to produce β -ketovaleryl-CoA; or

10

a β -ketothiolase capable of condensing two molecules of acetyl-CoA to produce acetoacetyl-CoA, and also capable of condensing acetyl-CoA and propionyl-CoA to produce β -ketovaleryl-CoA;

15

a β -ketoacyl-CoA reductase capable of reducing acetoacetyl-CoA and β -ketovaleryl-CoA to produce β -hydroxybutyryl-CoA and β -hydroxyvaleryl-CoA, respectively; and

20

a polyhydroxyalkanoate synthase capable of incorporating β -hydroxybutyryl-CoA and β -hydroxyvaleryl-CoA into P(3HB-co-3HV) copolymer;

wherein each of said introduced DNAs is operatively linked to regulatory signals that cause expression of said introduced DNAs; and

25

wherein said plant produces P(3HB-co-3HV) copolymer.

2. The plant of claim 1, the genome of which further comprises an introduced DNA encoding a wild-type or deregulated threonine deaminase

enzyme, wherein said introduced DNA is operatively linked to regulatory signals that cause expression of said introduced DNA.

3. The plant of claim 2, wherein said deregulated threonine deaminase is
5 *E. coli* threonine deaminase wherein leucine at amino acid position 447 is replaced with an amino acid selected from the group consisting of alanine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine.

4. The plant of claim 2, wherein said deregulated threonine deaminase is
10 *E. coli* threonine deaminase wherein leucine at amino acid position 447 is replaced with phenylalanine.

5. The plant of claim 2, wherein said deregulated threonine deaminase is
E. coli threonine deaminase wherein leucine at amino acid position 481 is
15 replaced with an amino acid selected from the group consisting of alanine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine.

6. The plant of claim 2, wherein said deregulated threonine deaminase is
E. coli threonine deaminase wherein leucine at amino acid position 481 is
20 replaced with phenylalanine.

7. The plant of claim 2, wherein said deregulated threonine deaminase is
E. coli threonine deaminase wherein leucine at amino acid position 447 is replaced with an amino acid selected from the group consisting of alanine,
25 isoleucine, valine, proline, phenylalanine, tryptophan, and methionine, and wherein leucine at amino acid position 481 is replaced with an amino acid selected from the group consisting of alanine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine.

30 8. The plant of claim 2, wherein said deregulated threonine deaminase is
E. coli threonine deaminase wherein leucine at amino acid position 447 is

replaced with phenylalanine, and wherein leucine at amino acid position 481 is replaced with phenylalanine.

9. The plant of claim 1, wherein said β -ketothiolase is *Alcaligenes eutrophus* BktB β -ketothiolase.

10. The plant of claim 1, wherein said β -ketoacyl-CoA reductase is obtainable from a microorganism selected from the group consisting of *Alcaligenes eutrophus*, *Alcaligenes faecalis*, *Aphanothece* sp., *Azotobacter vinelandii*, *Bacillus cereus*, *Bacillus megaterium*, *Beijerinckia indica*, *Dexia gummosa*, *Methylobacterium* sp., *Microcoleus* sp., *Nocardia corallina*, *Pseudomonas cepacia*, *Pseudomonas extorquens*, *Pseudomonas oleovorans*, *Rhodobacter sphaeroides*, *Rhodobacter capsulatus*, *Rhodospirillum rubrum*, and *Thiocapsa pfennigii*.

15 11. The plant of claim 1, wherein said polyhydroxyalkanoate synthase is obtainable from a microorganism selected from the group consisting of *Alcaligenes eutrophus*, *Alcaligenes faecalis*, *Aphanothece* sp., *Azotobacter vinelandii*, *Bacillus cereus*, *Bacillus megaterium*, *Beijerinckia indica*, *Dexia gummosa*, *Methylobacterium* sp., *Microcoleus* sp., *Nocardia corallina*, *Pseudomonas cepacia*, *Pseudomonas extorquens*, *Pseudomonas oleovorans*, *Rhodobacter sphaeroides*, *Rhodobacter capsulatus*, *Rhodospirillum rubrum*, and *Thiocapsa pfennigii*.

25 12. The plant of claim 1, wherein each of said introduced DNAs is further operatively linked to a transit peptide coding region capable of directing transport of said enzyme encoded thereby into a plastid.

30 13. The plant of claim 12, wherein said plastid is located in a seed of said plant.

14. A plant, the genome of which comprises introduced DNAs encoding the following enzymes:

5 a wild-type or deregulated threonine deaminase;

Alcaligenes eutrophus BktB β -ketothiolase;

10 acetoacetyl-CoA reductase; and

10 a polyhydroxyalkanoate synthase obtainable from a microorganism selected from the group consisting of *Alcaligenes eutrophus*, *Alcaligenes faecalis*, *Aphanothece* sp., *Azotobacter vinelandii*, *Bacillus cereus*, *Bacillus megaterium*, *Beijerinckia indica*, *Dexia gummosa*, *Methylobacterium* sp., *Microcoleus* sp.,
15 *Nocardia corallina*, *Pseudomonas cepacia*, *Pseudomonas extorquens*,
Pseudomonas oleovorans, *Rhodobacter sphaeroides*, *Rhodobacter capsulatus*,
Rhodospirillum rubrum, and *Thiocapsa pfennigii*;

20 wherein each of said introduced DNAs is operatively linked to a transit peptide coding region capable of directing transport of said enzyme encoded thereby into a plastid, and regulatory signals that cause expression of said introduced DNAs in seeds of said plant; and

25 wherein said plant produces P(3HB-co-3HV) copolymer in seeds thereof.

15. A method of producing P(3HB-co-3HV) copolymer, comprising growing said plant of claim 1 and recovering said P(3HB-co-3HV) copolymer produced thereby.

16. A method of producing P(3HB-co-3HV) copolymer, comprising growing said plant of claim 14 and recovering said P(3HB-co-3HV) copolymer produced thereby.

5 17. A plant cell containing P(3HB-co-3HV) copolymer.

18. A plant comprising cells containing P(3HB-co-3HV) copolymer.

10 19. The plant of claim 18, wherein said cells are located in leaves, stems, roots, or seeds of said plant.

15 20. The plant of claim 18, which is selected from the group consisting of soybean, canola, flax, and sunflower.

15 21. An isolated β -ketothiolase capable of condensing acetyl-CoA and propionyl-CoA to produce β -ketovaleryl-CoA.

20 22. An isolated β -ketothiolase capable of condensing acetyl-CoA and butyryl-CoA to produce β -ketocaproyl-CoA.

20 23. An isolated β -ketothiolase capable of:

 condensing two molecules of acetyl-CoA to produce acetoacetyl-CoA;

25 condensing acetyl-CoA and propionyl-CoA to produce β -ketovaleryl-CoA;

 and

 condensing acetyl-CoA and butyryl-CoA to produce β -ketocaproyl-CoA.

24. The isolated β -ketothiolase of claim 21, 22, or 23, which is *Alcaligenes eutrophus* BktB β -ketothiolase having the amino acid sequence shown in SEQ ID NO:11.

5 25. An isolated DNA molecule comprising a nucleotide sequence encoding said deregulated *E. coli* threonine deaminase of claim 3.

10 26. An isolated DNA molecule comprising a nucleotide sequence encoding said deregulated *E. coli* threonine deaminase of claim 5.

27. The isolated DNA molecule of claim 25, comprising the nucleotide sequence shown in SEQ ID NO:5.

15 28. The isolated DNA molecule of claim 26, comprising the nucleotide sequence shown in SEQ ID NO:8.

29. An isolated DNA molecule comprising a nucleotide sequence selected from the group consisting of:

20 (a) the nucleotide sequence shown in SEQ ID NO:9 or the complement thereof;

25 (b) a nucleotide sequence that hybridizes to said nucleotide sequence of (a) under a wash stringency equivalent to 0.5X SSC to 2X SSC, 0.1% SDS, at 55-65°C, and which encodes an enzyme having β -ketothiolase enzymatic activity similar to that of *A. eutrophus* BktB β -ketothiolase;

30 (c) a nucleotide sequence encoding the same genetic information as said nucleotide sequence of (a), but which is degenerate in accordance with the degeneracy of the genetic code; and

(d) a nucleotide sequence encoding the same genetic information as said nucleotide sequence of (b), but which is degenerate in accordance with the degeneracy of the genetic code.

5

30. An isolated DNA molecule, comprising the nucleotide sequence shown in SEQ ID NO:9 or the complement thereof.

31. A plant, the genome of which comprises introduced DNAs encoding
10 the following enzymes:

a β -ketothiolase capable of condensing two molecules of acetyl-CoA to produce acetoacetyl-CoA;

15 PhbB; and

PhbC;

wherein each of said introduced DNAs is operatively linked to a transit
20 peptide coding region capable of directing transport of said enzymes into a plastid, and regulatory signals that cause expression of said introduced DNAs in seeds of said plant; and

wherein P(3HB) homopolymer is produced in seeds of said plant.

25

32. The plant of claim 31, wherein said β -ketothiolase capable of condensing two molecules of acetyl-CoA to produce acetoacetyl-CoA is selected from the group consisting of PhbA and BlktB.

33. A method of producing P(3HB) homopolymer, comprising growing said plant of claim 31, and recovering said P(3HB) homopolymer produced thereby.

5 34. A plant, seeds of which contain P(3HB) homopolymer.

35. A seed containing P(3HB) homopolymer.

36. A method for transforming canola, comprising:

10

(a) preparing a stem explant from a canola plant by:

(i) removing leaves and buds along the stem and removing 4-5 inches of said stem below the flower buds; and

15

(ii) cutting said 4-5 inches of stem into segments;

20

(b) inserting DNA to be introduced into said explant of step (a) by inoculating said explant with a disarmed *Agrobacterium tumefaciens* vector containing said DNA;

25

(c) culturing said explant of step (b) in the basal-side down orientation;

(d) selecting transformed explant tissue; and

(e) regenerating a differentiated transformed plant from said transformed explant tissue of step (d).

30

37. A method for transforming soybean, comprising:

(a) preparing a cotyledon explant from a soybean seedling by:

100-200-300-400-500-600-700-800-900

(i) incubating said seedling at about 0°C to about 10°C for at least 24 hours;

5 (ii) removing the hypocotyl region by cutting in the region of from about 0.2 to about 1.5 cm below the cotyledonary node;

(ii) splitting and completely separating the remaining attached hypocotyl segment, also thereby separating the two cotyledons;

10 (iii) removing the epicotyl from the cotyledon to which it remains attached; and

(iv) wounding the cotyledon in the region of said axillary bud;

15 (b) inserting DNA to be introduced into said explant of step (a) by inoculating at least the region adjacent to the axillary bud of the explant with a disarmed *Agrobacterium tumefaciens* vector containing said DNA;

(c) selecting transformed explant tissue; and

20 (d) regenerating a differentiated transformed plant from said transformed explant tissue of step (c).

38. A soybean explant prepared by steps (a)(i)-(a)(iv) of claim 37.

25 39. Soybean tissue prepared from a seedling cotyledon pair containing an epicotyl, axillary buds, and hypocotyl tissue, comprising a single cotyledon containing an axillary bud and associated hypocotyl segment extending from about 0.2 to about 1.5 cm below the cotyledonary node;

30

wherein said associated hypocotyl segment is completely separated from its adjacent hypocotyl segment attached to the remaining cotyledon, thus separating said cotyledons;

5 wherein said epicotyl has been removed from the cotyledon to which it is attached;

wherein the cotyledon is wounded in the region of said axillary bud; and

10 wherein said seedling has been incubated at a temperature of from about 0°C to about 10°C for at least about 24 hours prior to preparing said soybean tissue.

40. A bacterium, the genome of which comprises introduced DNAs
15 encoding the following enzymes:

a wild-type or deregulated threonine deaminase;

20 a β -ketothiolase capable of condensing two molecules of acetyl-CoA to produce acetoacetyl-CoA and a β -ketothiolase capable of condensing acetyl-CoA and propionyl-CoA to produce β -ketovaleryl-CoA; or

25 a β -ketothiolase capable of condensing two molecules of acetyl-CoA to produce acetoacetyl-CoA, and capable of condensing acetyl-CoA and propionyl-CoA to produce β -ketovaleryl-CoA;

a β -ketoacyl-CoA reductase capable of reducing acetoacetyl-CoA and β -ketovaleryl-CoA to produce β -hydroxybutyryl-CoA and β -hydroxy-valeryl-CoA, respectively; and

a polyhydroxyalkanoate synthase capable of incorporating
 β -hydroxybutyryl-CoA and β -hydroxyvaleryl-CoA into P(3HB-co-3HV)
copolymer;

5

wherein said introduced DNAs are operatively linked to regulatory
signals that cause expression of said introduced DNAs; and

wherein said bacterium produces P(3HB-co-3HV) copolymer.

10

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

